Reducing Metal Contamination in Cu-64 production

S. Poniger[a,b], H. Tochon-Danguy[a], J. Sachinidis[a], K. Alt[c], C. Hagemeyer[c], A. Scott[a,b]

[a]*Centre for PET, Austin Health, Melbourne, Australia*
[b]*Ludwig Institute of Cancer Research, Melbourne branch, Australia*
[c]*Heart & Diabetes Institute, Baker IDI, Melbourne, Australia*

Introduction

In the past several years there has been a growing interest in the development of radiopharmaceuticals labeled with metallic radionuclides (1). Of particular interest is the positron emitter ^{64}Cu ($t_{½}$ = 12.7 h) for molecular imaging of small molecules as well as peptides and antibodies (2).

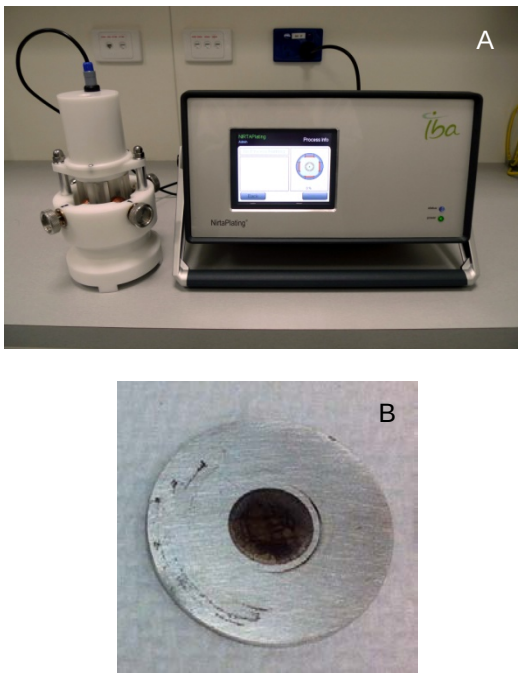
This has led us to the recent implementation of a solid target production facility using commercially available target irradiation station and chemistry modules. Using the ^{64}Ni(p,n)^{64}Cu nuclear reaction, routine production of ^{64}Cu was achieved with an average E.O.B. yield of 58.8 MBq (1.59 mCi)/μAh at 13.0MeV, however purification of ^{64}Cu has proven to be problematic; with several metallic contaminants compromising subsequent radiolabeling.

We report in this work, the step by step procedure which led us to the successful production of low metal contaminant ^{64}Cu with high specific activity and high labeling efficiency.

Material and Methods

Detailed implementation of our solid target was reported earlier (3). A Nirta Solid Target from IBA was coupled to our 18/9 cyclotron using a 2-meter external beam line. Typical irradiation parameters were 13.0MeV and 14.9MeV at 35 μA for 4-6 hours with ^{64}Ni platings ranging from 10-60 μm thickness and 6-12 mm∅. A pneumatic solid target transfer system (STTS) designed by TEMA was use to deliver the irradiated target disks to a dedicated hotcell. Two separate modules developed by IBA (Pinctada metal) were used for 1) electroplating ^{64}Ni onto a Ag disk and 2) acid dissolution and purification of the irradiated target material (see pictures 1 and 2).

1) Electroplating: Highly enriched (>99%) ^{64}Ni (100mg) was dissolved in 10mL 12M HCl (Sigma TraceSELECT) and refluxed for >8 hours under vacuum with gentle heating in a PFA round bottom flask until all ^{64}Ni had dissolved. Ag disks were prepared for electroplating by fine sanding, etched in 20% HNO_3 for 5min under ultrasound to dissolve any traces of oxides and then rinsed with D.I. water and acetone. The electroplating cell was filled with the ^{64}Ni solution (1-2mL) and completed to 55mL with NH_4OH. Electroplating of ^{64}Ni onto a 1mm thick 24mm diameter Ag disk was carried out at 3.0-5.0mA using a chopped saw tooth current for ~24 hours (Picture 1 A & B).



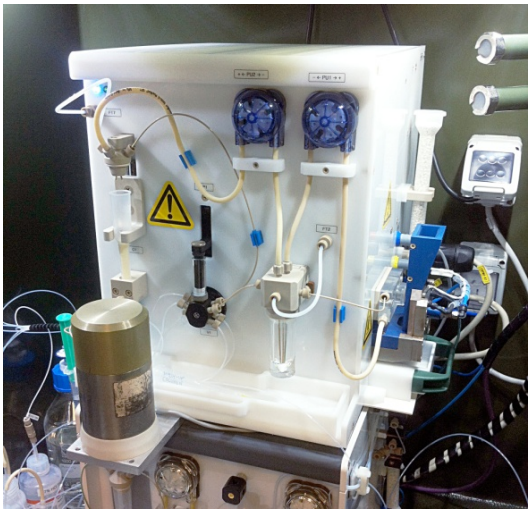
PICTURE 1. (A) Pinctada quad electroplating cell with controller and (B) Plated Ag disk (24mm∅) with 62μm (7mm∅) ^{64}Ni plating

2) Dissolution/purification: After irradiation, the target disk was left to decay for ~10 hours before processing to reduce the amount of co-produced short lived isotopes. The irradiated disk was then loaded into the Pinctada dissolution module (Picture 2) and the ^{64}Ni plating dissolved in recirculation 3mL 12M HCl at 70-80°C for 15-20 minutes.

The dissolved solution was then loaded onto an AG 1-X8 anion exchange cartridge and then washed with 4mL 6M and 2mL 4M HCl with the wash fractions collected separately to enable recycling of ^{64}Ni for further productions.

[1]Corresponding author, E-mail: stan.poniger@austin.org.au

^{64}Cu was eluted from the cartridge with 0.1M HCl in ~1.5mL.



PICTURE 2. 2nd Generation IBA Pinctada Metal dissolution and purification module inside Tema RES hotcell

Radionuclidic purities were evaluated by gamma spectroscopy with a high purity germanium detector (Canberra BEGe 2020) coupled to a multichannel analyzer (LYNX 32k) and the spectrum was analyzed using Canberra Genie 2000 software. In order to determine the specific activity of the samples, traces of metallic impurities were determined by ICP-MS or ICP-AES. Labeling efficiency was evaluated by measuring the amount of ^{64}Cu uptake per 20 µg of scFv-MeCOSar cage using thin layer chromatography (4).

Results and Conclusion

Initial ^{64}Cu purifications following the manufacturers recommended method show that despite ^{64}Ni being plated directly on an Ag disk, little Ag contamination was observed. However, high levels of Cu, Fe and Zn metal contaminants were observed (see Table 1, ID 1). After several productions, visual inspection of the module quickly revealed that the copper heater block used for heating the back of the Ag target disk may have been the cause of our contaminations as it was heavily corroded. Replacing the heater block with a PEEK heater block drastically reduced the levels of Cu and Fe contaminants.

Unfortunately, unusually high levels of Zn were still observed regardless of the stringent conditions and ultrapure reagents used during the processing (see Table 1, ID 5). In our quest for answers, ICP-MS analysis of the ^{64}Ni plating solution as well as critical stock reagents such as Milli-Q water (18 MΩ cm^{-1}) and 30% HCl Trace-Select Ultra (Sigma) was performed (see Table 1, ID 2,3,4). The results were surprising, with high level of Zn found not only in the ^{64}Ni plating solution, but as well in the HCl TraceSelect Ultra.

It has been reported in the literature (5,6) that most glass contains contaminating metals, including Zn that could leach into solution and we hypothesized that the Pinctada's glass bottles used to store the reagents, especially concentrated acidic solutions were the source of Zn contamination and all glass bottles were replaced by LDPE or PFA types. Our hypothesis was confirmed by subsequent ICP-MS analysis of fresh samples of HCl TraceSelect Ultra and the ^{64}Ni plating solution prepared/stored in plastic containers (see Table 1, ID 6,7). We also confirmed by ICP-MS analysis that no contamination occurred when performing a non-radioactive dissolution/purification sequence on the Pinctada module using a blank PTFE target disk in conjunction with the change to plastic reagent storage bottles (see Table 1, ID 8).

Initially the purification protocol was modified as described by Ometakova *et al*. (7) to help reduce the co-elution of Zn contaminants with the ^{64}Cu from the AG1-X8 resin. Unfortunately, this change resulted in a significant amount of ^{64}Cu eluting from the resin during the resin washing steps, so that protocol was abandonded and the protocol as described by Thieme *et al*. (8) was adopted. By modifying the AG1-X8 resin washing protocol and eluting the ^{64}Cu from the column with 0.1M HCl rather than Milli-Q water, we were able to further reduce metal contaminants especially Zn (see Table 1, ID 9).

Routine production of ^{64}Cu was achieved with an average E.O.B. production yield of 58.8 MBq (1.59 mCi)/µAh at 13.0 MeV. Typical irradiations at 13.0MeV and 36 µA for 3-4 hours resulted in the average recovery of 3.5 GBq (95 mCi) of purified ^{64}Cu at E.O.S.. ICP-MS/AES shows that during the course of these experiments, the true specific activity of ^{64}Cu increased from as low as 0.44 GBq/µmol (12 mCi/µmol) of Cu (n=2, Table 1, ID 1) to 24 GBq/µmol (649 mCi/µmol) of Cu (n=7, Table 1, ID 5) and finally to 148 GBq/µmol (4412 mCi/µmol) of Cu (n=3, Table 1, ID 9). In the same time, the effective specific activity increased from 1.1 ± 0.74 MBq (0.03 ± 0.02 mCi) per 20 µg of

[1]Corresponding author, E-mail: stan.poniger@austin.org.au

ID	Sample Description	ICP-MS / ICP-AES Results						S.A. Cu
		Cobalt (mg/L)	Copper (mg/L)	Iron (mg/L)	Nickel (mg/L)	Silver (mg/L)	Zinc (mg/L)	GBq/µmol (mCi/µmol)
1	Initial Cu-64 productions (avg.; n=2)	0.0215	5.5524	7.8471	0.9322	0.3199	19.3364	0.44 (12)
2	Stock reagent, 30% HCl TraceSelect Ultra (Sigma), stored in glass bottle	0.0008	0.0135	0.1125	0.0075	0.0203	21.7500	NA
3	Milli-Q water (18MΩ cm^{-1}), stored in glass bottle	0.0001	0.0001	0.0050	0.0010	0.0001	0.0060	NA
4	Ni-64 plating solution, prepared with/stored in glass bottle	0.0100	0.5000	0.5000	11000.0	0.6500	83.0000	NA
5	Cu-64 productions, replaced target disk heater block (Cu to PEEK) (avg.; n=7)	0.0101	2.3898	0.7159	1.0373	0.1078	40.7143	24 (649)
6	Stock reagent, 30% HCl TraceSelect Ultra (Sigma), stored in LDPE/PFA bottle	0.0008	0.0008	0.0375	0.0075	0.0458	0.0075	NA
7	Ni-64 plating solution, prepared with/stored in LDPE/PFA containers	0.0060	0.0180	0.3000	3.1800	0.4920	0.0600	NA
8	Pinctada module non-radioactive contamination test with blank PTFE target disk	0.0009	0.0891	0.2182	0.0182	0.0173	0.0909	NA
9	Cu-64 productions, LDPE/PFA reagent storage bottles, revised purification protocol (avg.; n=3)	0.0150	0.5450	0.8000	0.1500	2.3300	1.0000	148 (4412)

TABLE 1. ICP-MS metal analysis and corresponding specific activity of Cu

scFv-MeCOSar^{64}Cu before optimization, to 3.7 ± 0.3 MBq (0.1 ± 0.01 mCi) per 20 µg of scFv-MeCOSar^{64}Cu after optimalization of the procedures.

In conclusion, a significant reduction in Cu, Fe and Zn contaminants was achieved when processing ^{64}Cu using the Pinctada module: i) after replacement of the Cu heater block; ii) after elimination of glass reagent storage containers from the Pinctada module and procedures during preparation of the ^{64}Ni plating solution and iii) after implementation of a new purification protocol (8). Introduction of a 6M HCl wash-up cycle of the module prior to the dissolution procedure was also effective. However in recent ^{64}Cu productions slightly elevated Ag levels have been observed and are under investigation (see Table 1, ID 9).

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[1]Corresponding author, E-mail: stan.poniger@austin.org.au